Chapter

Post-Translational Regulation of the Activity of ERK/MAPK and PI3K/AKT Signaling Pathways in Neuroblastoma Cancer

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Abstract

Pathogenesis of cancer is a multi-step process containing a number of cellular alterations such as post-translational dysregulation of intracellular signaling proteins. These alterations control several functions in carcinogenesis such as angiogenesis, metastasis, evading growth suppressors, and sustaining proliferative signaling. Data of various studies has demonstrated that Phosphatidylinositol 3-kinase (PI3K/AKT) and Mitogen-activated protein kinase (ERK/MAPK) pathways are both abnormally activated in many cancer types, including neuroblastoma. ERK/MAPK and PI3K/AKT signaling pathways that are regulated by sequential phosphorylation upon extracellular stimulation have many important functions in cell cycle, migration, proliferation and apoptosis. Besides their aberrant phosphorylation/activation, there is a crosstalk between these two pathways resulting in an anti-apoptotic effect. In this chapter, carcinogenetic abnormalities in post-translational regulation of the activity of ERK/MAPK and PI3K/AKT pathways in neuroblastoma and other cancers will be summarized. In addition, several crosstalk nodes between two pathways will be briefly explained. All these concepts are not only crucial for thoroughly understanding the molecular basis of carcinogenesis but also choosing the appropriate molecular targets for effective diagnosis and treatment.

Keywords: Neuroblastoma, carcinogenesis, intracellular signaling, phosphorylation, ERK/MAPK, PI3K/AKT

1. Introduction

Cancer pathogenesis is mainly characterized by the accumulation of genetic, epigenetic, and post-translational alterations particularly in cellular signaling pathways leading to the manifestation of the cancer hallmarks such as enabling replicative immortality, sustaining proliferative signaling, activating invasion and metastasis, and inducing angiogenesis. Most of these alterations in the signaling pathways are observed on those that control cell growth, proliferation and death, cell fate and motility such as ERK/MAPK and PI3K/AKT pathways [1]. Under normal conditions, cell signaling process works as a regulated cascade, and as a result of these regulated signaling, healthy tissue structure is maintained, and cellular

functions are properly performed. However, in case of carcinogenesis, a multistep progress including various abnormalities in epigenetic and post-translational modifications of the components of these signaling pathways (e.g., acetylation, methylation, phosphorylation, ubiquitination, sumoylation etc.) occurs that triggers tumorigenic growth [2].

In carcinogenesis, three types of gene groups, oncogenes, tumor-suppressor genes, and stability genes are the primary sources for oncogenic mutations. As a result of these mutations, genes are over-expressed/silenced, or mutated proteins with dysregulated functions are produced. However, examining the carcinogenesis in detail revealed that mutated genes are not the only responsible for cancer development, and hence focusing on intracellular signaling pathways rather than individual genes is more significant. Several mutations may also be observed in different components of these signaling pathways, and most of these mutations are known to be common for different cancer types [3].

On the other hand, ERK/MAPK and PI3K/AKT signaling pathways have cross talking nodes which post-translationally affect their activity and control many of the important cellular functions such as cellular metabolism, cell growth, division, death, differentiation, and movement. However, this crosstalk becomes severely disturbed in many cancers, including neuroblastoma, resulting in rapid disease progression and poor prognosis [4–6].

Neuroblastoma is one of the most common pediatric cancers that arises from immature sympathetic nervous system precursors and localizes in adrenal gland or sympathetic ganglia [7]. Neuroblastoma tumors have a very high degree of heterogeneity, ranging from more favorable to highly aggressive tumors with high lethality. In neuroblastoma, as in many types of cancer, ERK/MAPK and PI3K/AKT pathways in particular are notable in terms of their contribution to oncogenic transformation and severity of the disease [8, 9].

Therefore, in this chapter, function of ERK/MAPK and PI3K/AKT signaling in different cancers, as well as in neuroblastoma will be summarized. Then their aberrant and oncogenic interaction with each other and with other cellular components will be discussed.

2. ERK/MAPK signaling pathway in cancer

ERK/MAPK is a highly conserved signaling pathway in the evolutionary process that provides signal transduction via Receptor Tyrosine Kinases (RTKs). MAPKs regulate important cellular functions (e.g., cell cycle, proliferation, migration etc.) through phosphorylation of specific serine/threonine regions of target proteins [10, 11]. Four MAPK cascades have been identified in mammalian cells: Extracellular signal-regulated kinase (ERK, classical MAPK), C-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), p38 MAP kinase, and ERK5 [12]. Among them, ERK/MAPK is the most important signaling cascade in tumor development. MAPK enzymes in all eukaryotic cells are found at the junctions of mitogenic stimuli received by different receptors. In response to received stimuli, the signal is transferred intracellularly to the small oncogenic G-protein Ras and then to the Raf (MEK kinase) protein. Activated Raf protein activates MEK1/2 (MAPK/ERK kinase or MAP kinase kinase) signal protein which then phosphorylates and activates ERK1/2 ultimately regulating essential cellular events such as gene expression, mitosis, cell viability, apoptosis, motility, differentiation, and cellular metabolism [13].

In addition to phosphorylation/dephosphorylation, positive and negative regulation of ERK/MAPK signaling involves other types of post-translational modifications as well. Among them, post-translational modifications of Ras protein such

as farnesylation and methylation are intriguing for providing fully active Ras. Ras protein activation requires a serial post-translational modifications that allow Ras to localize to the plasma membrane [14]. The first post-translational modification is farnesylation of the Ras carboxylterminal peptide CAAX through covalent binding of a farnesyl isoprenoid lipid by cytosolic farnesyltransferase enzyme. Then, Ras converting enzyme 1 (Rce1) cleaves AAX tripeptide to generate a free cysteine residue. Finally, a methyl group is covalently attached to this cysteine residue by isoprenylcysteine-O-carboxyl methyltransferase (ICMT) to facilitate the transfer of Ras to the plasma membrane [15].

On the other hand, ERK/MAPK signaling may be downregulated by post-translational modifications including ubiquitination, SUMOylation, and acetylation/deacetylation reactions, In a study, it was shown that Ras- or MEK-mediated ERK activation is attenuated by SIRT1 through deacetylation of the dual specific phosphatase for MAPK, MKP1 which results in the inhibition of cellular proliferation and transformation. They revealed that binding affinity of deacetylated MKP1 to ERK is increased subsequently leading to the inactivation of ERK [16].

In addition, ERK1/2 protein may be inactivated by its degradation through Ubiquitin-Proteasome System (UPS). This process is mediated by an upstream MAP3K, MEKK1 bearing ubiquitin ligase activity, that triggers ERK ubiquitination [17, 18]. Another member of ERK/MAPK pathway, c-Raf, is also degraded by the UPS under certain conditions. Hsp90 is a chaperone responsible for the stability and function of c-Raf protein, and Hsp90 degradation promotes destabilization and degradation of c-Raf by UPS. In a study, it was identified that the antitumor effect of the antibiotic benzoquinone ansamycin geldanamycin is a result of its binding to Hsp90 which triggers its degradation [19].

Fırthermore, the ERK/MAPK signaling may be downregulated by SUMOylation of MEK (MAPK–ERK kinase). SUMOylated MEK loses its interacting ability with ERK which ends up with the blocking of ERK activation. Oncogenic Ras prevents this process in carcinogenesis by disrupting the SUMO E3-ligase activity of MEKK1 MAPKKK [20].

The ERK/MAPK signaling pathway is one of the main oncogenic pathways and overactivated approximately 30% of human tumors [21]. The ERK/MAPK pathway, particularly when activated by growth factors and mitogens has the strongest correlation with cancer. Carcinogenic abnormalities including over-expressing/activating mutations of RTKs, constant production of activating ligands, and Ras/Raf mutations trigger the continuous activation of the ERK/MAPK pathway which indicates that carcinogenic dysregulation of this pathway may occur at different levels (**Table 1**) [24].

There is a large body of evidence describing the contribution of ERK/MAPK signaling to cancer progression. In a study, it was shown that the expression of MKP-1 which is the negative nuclear regulator of ERK/MAPK signaling is increased in normal ovarian surface epithelium and benign cystadenomas compared to invasive carcinomas and tumors with low malignancy potential and borderline tumors. The level of MKP-1 expression in tumor tissues of patients with stage III/IV disease was found to be significantly lower compared to patients with stage I/II disease which is in contrast to the results indicating a significantly higher expression of phosphorylated-ERK1/2 (p-ERK1/2) in stage III/IV tumors compared with that in stage I/II tumors. These data point out to a negative correlation between MKP-1 and p-ERK1/2 expression in the same ovarian cancer tissue which emphasizes the significance of the abnormal expression of MKP-1 and its effect on ERKs phosphorylation in the development of ovarian cancer [25].

Moreover, in another study with colon tissue, they showed that in colon cancer, tubular adenoma, and villous adenoma, MEK phosphorylation rates were 76, 30

Type of mutations in MAPK signaling pathway	Rate of mutations in different cancers
EGFR **over-expression**	Most carcinomas (>50%)
ERBB2 **over-expression**	Breast (30%)
	Pancreas (90%)
	Lung adenocarcinoma (35%) (non-small cell)
	Thyroid; follicular (55%)
	Thyroid; undifferentiated papillary (60%)
RAS **mutation**	Seminoma (45%)
	Melanoma (15%)
	Bladder (10%)
	Liver (30%)
	Kidney (10%)
	Myelodysplastic syndrome (40%)
	Acute myelogenous leukemia (30%)
BRAF **mutation**	Melanoma (66%)
	Colorectal (12%)
MEK **or** *ERK* **mutation**	Melanoma (3–8%)
	Colorectal (3%)

Table 1.
Mutation rates in MAPK signaling pathway of different cancers [22, 23].

and 40%, respectively. However, the phosphorylation of MEK in normal colonic mucosal cells was scarcely detectable [26]. In addition, in a study examining ERK/MAPK pathway's function in cellular growth and differentiation in colon carcinoma of mice by Sebolt-Leopold et al., oral intake of MEK inhibitor provide inhibition of tumor growth in the rate of 80% [27].

As in other types of cancer, aberrant signaling of ERK/MAPK pathway is crucial for neuroblastoma cancer since it leads to reduced therapeutic efficacy [28]. Nevertheless, there is limited number of studies focusing on the role of abnormal ERK/MAPK signaling in neuroblastoma cancer. In one study, it was shown that this signaling pathway is responsible for the transformation of neuroblastoma cells and gaining resistance to chemotherapy [29]. In this study, they incubated SKNSH neuroblastoma cell lines with increasing concentrations of doxorubicin or MDL 28842 for long-term to establish drug resistant SKNSH cell lines. Then they analyzed the levels of epidermal growth factor receptor (EGFR) expression and epidermal growth factor (EGF)-induced EGFR tyrosine phosphorylation and determined that they were both lower in drug-resistant SKNSH cells compared with their wild-type counterparts. In addition, in doxorubicin treated SKNSH cells, MAPK activation and nuclear translocation were found to be decreased in response to EGF. These results reveal that chemotherapeutic drug resistance in human neuroblastoma cell lines is in close association with low levels of growth factor signaling through the MAPK pathway.

While continuous activation of the ERK/MAPK signaling pathway promote the transformation of normal cells into tumor cells, inhibition of the ERK/MAPK signaling can restore tumor cells to their non-transformed state in vivo and in vitro [27]. In our laboratory, we examined the effect of ERK/MAPK inhibition on the SH-SY5Y neuroblastoma cell viability. Results of MTS cell viability analysis showed that the

viability of SH-SY5Y neuroblastoma cells upon treatment with the specific MEK1/2 inhibitor U0126 was significantly decreased. This result indicates a close link between ERK/MAPK pathway and carcinogenesis in SH-SY5Y neuroblastoma cells [30].

On the other hand, iron chelators have also been used to inhibit the ERK/MAPK signaling pathway and it has been shown in prostate cancer cells that they are able to regulate the ERK/MAPK signaling by reducing ERK1/2 phosphorylation [13, 31]. Based on the results of these studies, we examined the anti-proliferative effects of iron-chelating salicylidene acylhydrazide group synthetic compounds ME0053, ME005 and ME0192 in SH-SY5Y neuroblastoma cells by analyzing the effects of these compounds on ERK/MAPK and PI3K/AKT activities. The results indicated that these iron-chelators caused a significant decrease in MEK1/2 expression and AKT phosphorylation in neuroblastoma cells [24]. These results are promising for alleviation of the ERK/MAPK activation by utilizing different iron chelators to prevent cancer.

Furthermore, current studies have shown that non-steroidal drugs containing salicylic acid (SA) decrease mortality through mitogenic MEK1/2 protein, an important member of ERK/MAPK signaling in many cancers putting a spotlight on SA as a potential inhibitor of MEK1/2 signaling in the prevention of carcinogenic progression [32–34]. In our laboratory, we studied with a salicylic acid analog acibenzolar-S-methyl to analyze its effects on MEK1/2 signaling in SH-SY5Y neuroblastoma cells and we showed that acibenzolar-S-methyl negatively affects MEK1/2 signaling causing apoptotic death of SH-SY5Y neuroblastoma cells [35]. Besides, in one study conducted with A549 human lung cancer cells, SA has been demonstrated as a suppressor of this vital signaling pathway by inhibiting the binding of c-Raf to Ras protein, disrupting phosphorylation state of c-Raf and thereby damaging the MAPK signaling [36]. The great number of protein kinases modulated by salicylate may be explanatory for the question 'what is the apoptotic mechanism of salicylate in cancer?' [36, 37].

Moreover, in an effort to suppress ERK/MAPK signaling in neuroblastoma, Woodfield et al. [38] hypothesized that inhibiting ERK/MAPK signaling through the novel MEK1/2 inhibitor binimetinib may be effective in neuroblastoma models. For this purpose, they analyzed the response of binimetinib-sensitive and binimetinib-resistant neuroblastoma cells from tissue samples and neuroblastoma cell lines by examining total and phosphorylated MEK and ERK levels. They demonstrated that both primary neuroblastoma tumor cells and cell lines showed significant levels of total and phosphorylated MEK and ERK, while binimetinib treatment caused complete loss of phosphorylated ERK. However, resistant cells showed negligible effects on ERK and MEK phosphorylation. They also showed that Ras-GTPase activating protein (RasGAP) NF1 expression was in correlation with responses to binimetinib, suggesting a potential role for NF1 and ERK/MAPK signaling in neuroblastoma differentiation, drug resistance and relapse [38].

Even though it is well known that ERK/MAPK signaling inhibition results in apoptotic death in many cancers, in certain types of cancer such as melanoma, suppressing this signal may inversely contribute to cancer formation by creating an anti-apoptotic effect [37]. This contradiction draws attention to the heterogeneous and unique nature of the molecular basis of cancer emphasizing the vitality of thoroughly understanding of molecular and cellular mechanisms of each cancer type.

3. PI3K/AKT signaling pathway in cancer

Similar to the ERK/MAPK pathway, PI3K/AKT signaling pathway is activated by the interaction of a growth factor with a RTK that regulates basic cellular functions

such as growth, proliferation, cellular metabolism, cytoskeletal organization, survival and apoptosis in normal cells [39]. PI3K, is a member of lipid kinase, is divided into three classes: classes I, II, and III according to its specific substrates and structures. The Class I PI3Ks which are composed of p55 and p85 regulatory subunits (p85a, p55a, p50a, p85b, p55g) and p110 catalytic subunit (p110a, p110b, p110d) is the most frequently associated class with cancer [40]. In normal cellular conditions, PI3K is activated by growth factors, cytokines, and hormones. Following this activation, PI3K triggers the phosphorylation reaction of PtdIns (4,5) P2(PIP2) to produce PtdIns (3,4,5) P3(PIP3).

The most important downstream effector protein of PI3K is a serine/threonine kinase AKT/protein kinase B (PKB) that regulates several mechanisms in cell survival and cell cycle progression [41]. In order to activate AKT signaling, the AKT protein is subjected to successive phosphorylation through Thr308 and Ser473 residues. Semi-active form of AKT protein is achieved by Thr308 phosphorylation, while a sequential phosphorylation on Ser473 region at the C-terminal end by PDK2 (phosphoinositide dependent protein kinase 2) leads to full activation of AKT. Activated AKT leaves the membrane and translocates to the cytoplasm and nucleus. Here, by phosphorylating a wide range of target proteins such as MDM2, mTOR, GSK3β and BAD, it causes cellular responses such as cell proliferation, survival, growth, DNA repair and suppression of apoptosis [28].

The negative regulator of the PI3K/AKT signaling pathway is the Phosphatase and Tensin homolog protein (PTEN), which has been defined as a tumor suppressor and is frequently affected by mutations in cancers. PTEN's substrate is PIP3, one of the PI3K products. PTEN inhibits the PI3K/AKT pathway activity by reducing the amount of PIP3, converting PIP3 back to PIP2 via dephosphorylation [42]. PTEN stability and activity is post-translationally regulated by Protein Inhibitor of Activated STAT xα (PIASxα) which is a SUMO E3 ligase for PTEN. PIASxα SUMOylates and stabilizes PTEN protein, thereby negatively regulates PI3K/AKT signaling and leads to G0/G1 cell cycle arrest, and cell proliferation inhibition [43].

Moreover, ubiquitination is another way of post-translational regulation of PI3K/AKT pathway. p85 subunit of PI3K is ubiquitinated and degraded by trosine-phosphorylated c-Cbl E3 ligase which ultimately leads to downregulation of PI3K/AKT signaling [44, 45]. Besides p85 ubiquitination, both caspase- and proteasome-dependent AKT degradation may downregulate PI3K/AKT signaling in case of vascular endothelial growth factor (VEGF) deprivation, mTOR inhibition, or TNF-α treatment [46, 47].

AKT signaling controls metabolic processes either directly, by regulating metabolic enzymes through phosphorylation, or indirectly, by regulating a number of transcription factors. Metabolic enzyme phosphorylation provides short-term changes in the metabolic pathways, while controlling gene expression through the phosphorylation of transcription factors allows for longer-term changes in intracellular metabolic pathways.

Even though AKT is primarily a survival kinase, it also enhances cell proliferation. Cyclin D-1 is a cell cycle regulator which is responsible for G1 to S phase progression. GSK3β phosphorylates cyclin D-1, enabling its transport from nucleus to the cytoplasm for degradation and thereby inhibiting cell cycle. AKT triggers cell proliferation not only by inhibiting this GSK3β kinase activity through phosphorylation, but also by downregulating cyclin dependent kinase inhibitors KIP1 (p27) and CIP1 (p21) [48–50].

Besides cell proliferation, the PI3K/AKT pathway has also been shown to be functional in physiologic and pathologic angiogenesis in animal models [51, 52]. In tumors, PI3K/AKT pathway exerts its pro-angiogenic effects through upregulating HIF-1α, thereby activating VEGF [53]. HIF-1, a heterodimeric protein with α and β

subunits, is an activator of VEGF transcription [54]. Moreover, there is data indicating a HIF-1α-independent pathway for PI3K-mediated VEGF upregulation through phosphorylation and activation of endothelial nitric oxide synthase by AKT [55, 56].

Aberrant regulation and activation of the PI3K/AKT pathway is frequent in numerous human malignancies playing a pivotal role in both cancer progression and drug resistance. PI3K/AKT activation is mainly a consequence of the loss of tumor suppressor gene PTEN [57, 58], oncogenic activation of PIK3CA [59, 60] and over-activation by a number of growth factors such as IGF-1, VEGF or EGF [61–63].

Loss-of-function mutations in the PTEN gene are extremely common among sporadic glioblastomas, melanomas, prostate cancers, and endometrial carcinomas. PTEN is negative regulator of the PI3K/AKT signaling pathway that dephosphorylates PIP3. Mutated PTEN leads to increased level of PIP3 that trigger continuous phosphorylation of AKT, thereby leading to continuous activation of the PI3K/AKT signaling pathway. Hyper-activated AKT promotes the survival of cancer cells by causing increased level of cell proliferation and resistance to apoptosis [64, 65].

Although it is obvious that PI3K/AKT also contributes to development of neuroblastoma, its molecular mechanism is poorly understood. Johnsen et al. [66] suggested a link between PI3K/AKT pathway and neuroblastoma through over-activated AKT which appears to be closely related to the disease outcome. In other studies, PI3K/AKT pathway activation was identified as a predictor of poor outcome in neuroblastoma, supporting the afore-mentioned study results and making it a clinically important therapeutic target [67–69]. In one of these studies, they analyzed the effect of small molecule PI3K inhibitors on chemosensitivity in neuroblastoma cell lines and primary cultured neuroblastoma samples. The results of the study showed that PI3K inhibitors, (PI103 for this study), work synergistically with certain chemotherapeutics (Doxorubicin, Etoposide, Topotecan, Cisplatin, Vincristine and Taxol) to drive neuroblastoma cells through apoptosis. PI103 elicits this function by cooperating with chemotherapeutics to decrease the PI3K-mediated inhibitory phosphorylation of pro-apoptotic BimEL, thereby turning the situation in favor of pro-apoptotic Bcl-2 proteins to trigger apoptosis. Thus, targeting PI3K/AKT presents a promising strategy to sensitize neuroblastoma cells for chemotherapy-induced apoptosis [67].

On the other hand, in a study with a murine model of neuroblastoma, they showed that inhibiting PI3K/AKT signaling prevents tumor progression through an effect on oncogenic Mycn protein stability by inactivating GSK3β [70]. Furthermore, AKT phosphorylation has been detected in different neuroblastoma cell lines such as SK-N-SH, SH-SY5Y, SK-N-BE, SH-EP, and IMR-32. Studies related with neuroblastoma cell lines revealed that activated AKT cause poor prognosis and the use of inhibitors specific to the PI3K/AKT signaling pathway leads cancer cells to apoptosis [67, 71]. In another study, SH-SY5Y neuroblastoma cells were exposed to interferon-β resulting in the downregulation of AKT and subsequent apoptosis [72].

4. Pathological interaction of ERK/MAPK and PI3K/AKT signaling pathways in neuroblastoma

Although the usual signaling networks of hormone, cytokine, and growth factor receptors present PI3K/AKT and ERK/MAPK as two independent pathways, there are several inter-pathway cross talk nodes as well as certain regulatory molecules that can simultaneously act on both pathways which together determine the fate of the cell [13, 31]. Based on this information, it can be stated that it is possible for the PI3K/AKT and ERK/MAPK pathways to affect each other either negatively or positively at different signal propagation stages (**Figure 1**).

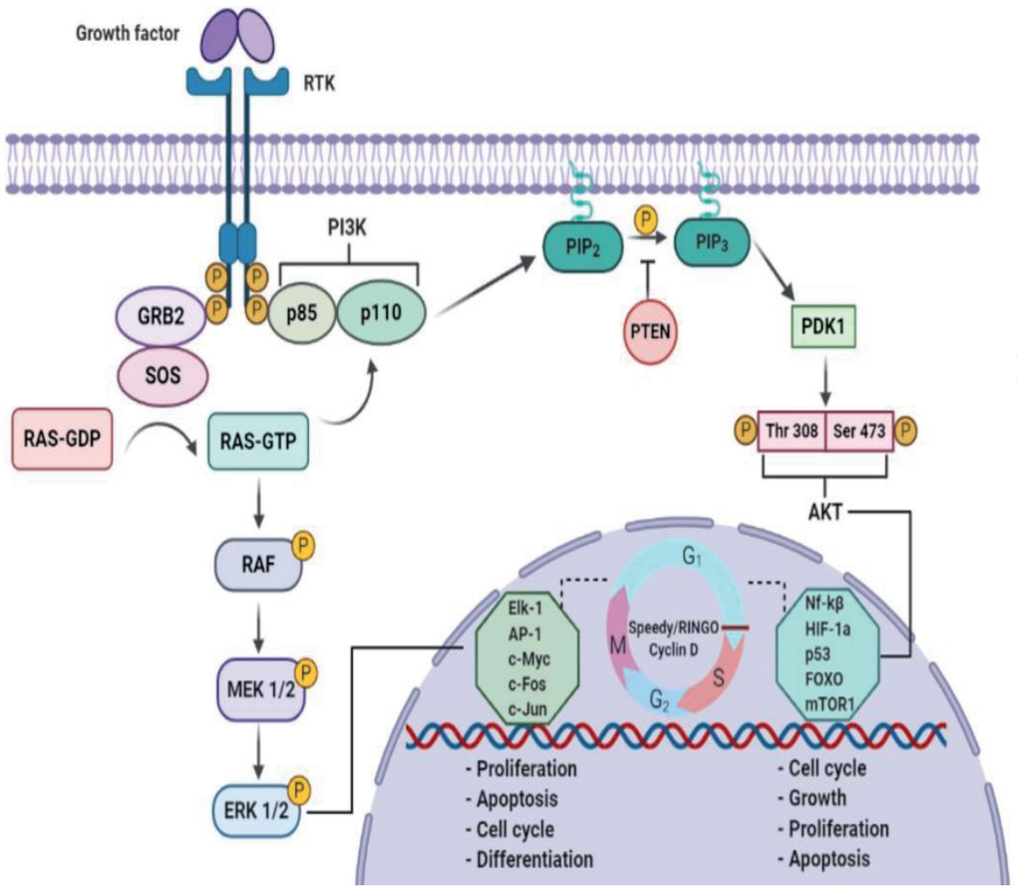

Figure 1.
Interactions of PI3K/AKT and ERK/MAPK pathways.

Examining the mutual talking points of these signaling pathways reveals that the activated RAS protein appears to have a binary switch function triggering both ERK/MAPK and PI3K/AKT pathways [61, 73]. Another important cross talk is the induction of Raf and MEK by PI3K. Cross talk interactions caused by PI3K activation and mediated by PDK1 activate the ERK/MAPK pathway, while AKT and downstream effectors, mTOR and p70S6K, negatively affect ERK/MAPK signaling [28]. On the other hand, active ERK can influence the PI3K/AKT pathway in different interaction routes. One mechanism involves modulation of the ERK-mediated phosphorylation levels at serine and threonine residues of certain AKT members (**Figure 1**) [74]. Moreover, Tuberous Sclerosis Complex 2 (TSC2) can be phosphorylated by either PI3K/AKT pathway or ERK/MAPK pathway, that allows for mTOR activation which is a component of PI3K/AKT signaling [75].

Investigating their interaction in terms of carcinogenesis, it is apparent that their abnormal interaction has an incontrovertible effect particularly on the aggressive progression of different types of cancers. In one of the studies examining their interaction in cancer, the role of the ERK/MAPK pathway in the control of self-renewal and tumorigenicity of glioblastoma cancer stem-like cells (CSLCs) was investigated in relation to the PI3K/AKT pathway. When they inactivated MEK1/2 using chemical inhibitors or siRNA, both cell line- and patient derived glioblastoma CSLCs were shown to lose their spherical form and differentiate into neuronal and glial lineages. Further, this observed effect of MEK inactivation was enhanced by using a dual inhibitor (NVP-BEZ235) of PI3K and mTOR suggesting that inactivating either ERK/MAPK or PI3K/AKT pathway leads to activation of the other, implying the presence of a mutual inhibitory cross talk between them [4].

In another study emphasizing the importance of concomitant inhibition of both pathways in terms of preventing the contributing effect of their cross talk on carcinogenic progression, a mouse model co-expressing the activated forms of AKT and Ras in the liver of mouse was utilized. They showed that continuously and simultaneously activated ERK/MAPK and PI3K/AKT signaling causes accelerated liver tumor development through activation of mTOR. In order to reveal the exact role of mTOR activation in AKT/Ras induced hepatocellular carcinoma, they treated AKT/Ras mice with mTOR inhibitor Rapamycin, and they found out that Rapamycin significantly prevented tumor formation in the liver of AKT/Ras mice. However, Rapamycin withdrawal resulted in rapid recurrence of hepatocellular carcinoma arising from the residual lesions in the liver of AKT/Ras mice through upregulation of ERK and its downstream effectors, Mnk1 and eIF4E in the lesions. Simultaneously suppressing PI3K/AKT and ERK/MAPK pathways was shown to significantly inhibited the growth of AKT/Ras cells in vitro, indicating that there is apparently a sophisticated interaction between these two pathways [5].

As in glioblastoma and hepatocarcinoma, cross talk between the PI3K/AKT and ERK/MAPK pathways contribute to carcinogenesis in rhabdomyosarcoma which is a rare cancer type of soft tissue. Since there is a multifaceted cross talk and a reciprocal compensation between them, blocking both pathways concomitantly in order to have a synergistic inhibitory effect on rhabdomyosarcoma progression was found to be more effective both in vivo and in vitro [6].

Although the effect of their aberrant cross talk is evident in many cancers, there is not sufficient number of studies conducted with neuroblastoma cell lines or primary neuroblastoma cells to investigate their oncogenic interaction in this cancer. On the other hand, ERK/MAPK and PI3K/AKT pathways, in addition to the interactions between their different components, can be affected by other proteins that are not members of these pathways [21]. However, the effect of these interactions on the emergence of cancer, especially of neuroblastoma, is not yet known. At this point, there are data that strengthen the possibility that one of the proteins likely to have an effect on PI3K/AKT and ERK/MAPK signaling pathways is the Speedy/RINGO protein, which is an unconventional cell cycle regulator and plays a very important role in many cancers [76].

Speedy/RINGO binds to its partner Cyclin-dependent kinase 2 (CDK2) and controls G1-S phase transition in the cell cycle [73]. In order to elicit this function, unlike classical cyclins, Speedy/RINGO does not require phosphorylation, and it is also resistant to phosphorylating inhibition by cell cycle inhibitors such as p21Cip1 and p27Kip1 [24]. Because of these properties, Speedy/RINGO can inhibit apoptosis and sustain cancerous cell division by overriding many cell cycle checkpoints [77, 78]. There are various studies conducted with neuroblastoma and breast cancer cells showing the contribution of Speedy/RINGO over-expression together with its partner CDK2 to the carcinogenic process [79, 80].

Apart from these studies, Speedy/RINGO protein has also been shown to have an interaction with ERK/MAPK signaling pathway in a study investigating the tumor formation in breast tissue [81]. In this study, they determined that activating ERK/MAPK pathway resulted in Speedy/RINGO over-expression, and inhibiting this signaling decreased Speedy/RINGO expression. Besides, with another study, it was shown that Speedy/RINGO over-expression leads to the increased activity of its partner proteins, CDK2 and Cyclin A [82]. On the other hand, studies with mouse embryonic stem cell indicated that the Cyclin A2 and CDK2 take part in AKT over-phosphorylation and activation (**Figure 1**) [83].

Considering the results of all these studies has led us to raise the question that "could there be an either direct or indirect three-way interaction between these

three players, Speedy/RINGO, ERK/MAPK and PI3K/AKT pathways in neuroblastoma cells?". To analyze this interaction, Speedy/RINGO gene expression was silenced by siRNA in SH-SY5Y neuroblastoma cells in order to determine its effect on the activity of ERK/MAPK and PI3K/AKT pathways. Results showed that silencing Speedy/RINGO in neuroblastoma cells significantly decreased MEK1/2 expression in ERK/MAPK pathway, and AKT Thr308 and Ser473 phosphorylations in PI3K/AKT pathway. Afterwards, ERK/MAPK signaling was blocked by a specific MEK1/2 inhibitor (U0126) in order to examine the effect of ERK/MAPK inhibition on Speedy/RINGO expression and PI3K/AKT signaling activity in SH-SY5Y cells. As a result, inhibiting ERK/MAPK signaling significantly reduced the expression of Speedy/RINGO and its partners CDK2 and Cyclin A as well as AKT phosphorylation suggesting a reciprocal interaction between Speedy/RINGO and ERK/MAPK and PI3K/AKT signaling pathways [30].

As previously mentioned in this chapter, iron homeostasis is in close relation with the regulation of ERK/MAPK and PI3K/AKT signaling activity. There are a growing number of studies demonstrating the strong effect of iron chelators on these two pathways particularly in prostate cancer [13, 31, 84]. Based on the results of these studies, we have investigated the effects of iron-chelating salicylidene acylhydrazide compounds (ME0053, ME0055 and ME0192) on the ERK/MAPK, PI3K/AKT pathways as well as on Speedy/RINGO expression for the reason that it is likely to be one of the effectors of these two pathways [24]. In this study, it was observed that both MEK1/2 activity and AKT phosphorylation on Thr308 and Ser473 sites were decreased together with a significant decrease in Speedy/RINGO expression emphasizing the effect of different metabolic processes such as iron homeostasis on the post-translational regulation of the members of these two pathways as well as on their interaction with other effector molecules such as Speedy/RINGO.

5. Conclusions

ERK/MAPK and PI3K/AKT pathways are very striking in terms of their contribution to carcinogenesis in many cancers. In this chapter, we have summarized the function of abnormal ERK/MAPK and PI3K/AKT signaling and their cross talk in cancer with an emphasis on neuroblastoma, and discussed their provoking action on the onset, progression, and severity of the disease. All afore-mentioned studies in this chapter will pave the way for better understanding of the aberrant post-translational regulation of oncogenic ERK/MAPK and PI3K/AKT pathways with an ultimate effort for fine-tuning of treatment modalities for cancer.

Acknowledgements

Studies mentioned here that were performed in our laboratory was supported by grants to Aysegul Yildiz from Mugla Sitki Kocman University Scientific Research Project Office, Research and Development Projects (Project Numbers: 17/251 and 17/023).

Conflict of interest

Authors declare that there is no conflicts of interest.

Thanks

We sincerely thank Prof. Dr. Uygar Halis Tazebay from Gebze Technical University, Department of Molecular Biology and Genetics and Prof. Dr. Arzu Karabay Korkmaz from Istanbul Technical University, Faculty of Science and Letters, Molecular Biology and Genetics Department for allowing us to use their laboratory infrastructure. We would also thank Assoc. Prof. Emin Ilker Medine from Ege University Institute of Nuclear Sciences for his help about providing SH-SY5Y neuroblastoma cell line, Prof. Dr. Mikael Elofsson from Umeå University for providing all the salicylidene acylhydrazide compounds, and Prof. Dr. Omur BAYSAL from Mugla Sitki Kocman University for his generous gift of acibenzolar-S-methyl (Syngenta).

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